Major Metabolites of Ginseng Sapogenins formed by Rat Liver Microsomes

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Incubation of ginseng sapogenins with microsomes from rat liver resulted in formation of their 20,24-epoxides as major metabolites. Identification of the metabolites was performed by HPLC, FAB-MS and EI-MS.

Key words microsomal metabolite; ginsenoside; 20(S)-protopanaxatriol; 20(S)-protopanaxatriol oxide; 20(S)-protopanaxadiol; 20(S)-protopanaxadiol oxide

Ginseng (the roots of *Panax ginseng* C. A. Meyer) is a famous oriental crude drug and has been used as a tonic for enhancing body strength, recovering physical balance and stimulating metabolic function. A number of saponins called ginsenosides have been isolated,1) and are regarded as the main biological active principles of ginseng. Most of these compounds were dammarane type triterpene saponins, and are classified into two groups according to their sapogenins, 20(S)-protopanaxatriol (1) and 20(S)-protopanaxadiol (2) (Fig. 1). Many studies on absorption, distribution and excretion of ginsenosides after oral administration have been reported.2)–4) These cumulative findings revealed that the uptake of ginsenosides occurs after complete or partial hydrolysis to yield sapogenins such as 1 and 2 or prosapogenins by the acidic conditions of stomach and intestinal flora. However, details on the metabolic pathway of the above primary metabolites after absorption from the digestive tract are not known.

It is believed that 1 and 2 having 24,25-double bond would be oxidized to 24,25-epoxide as one of the metabolites by liver enzymes such as microsomal cytochrome P450 acting as a monoxygenase. The oxidation of 1 or 2 with organic peracids leads to its 20-hydroxy-24,25-epoxy-compound. However, these compounds are usually unstable because of the existence of 20-hydroxy group and are immediately transformed to their 20,24-epoxides, and this biotransformation appears to be important in the pharmacological activities of ginsenosides. As basic research on the study of an active form of ginsenosides, we investigated the metabolism of the sapogenins by rat liver 9000 g supernatant (S9) fraction which consists of both microsomal and cytosolic fractions.

Experimental

Compounds 1 and 2 were obtained by alkaline hydrolysis of ginsenosides Re and Rb1, respectively, according to the method described in the literature.1) Reference standards 3 and 4 from 1, and 5 and 6 from 2 were prepared by oxidation with m-chloroperbenzoic acid.6) All chemicals were of reagent grade. EI- and FAB-MS were recorded on a JEOL JMS-SX-102 spectrometer. HPLC was performed on a JASCO PU-980 pump with a TOYO SODA R1-8000 detector using octadecyl silicic (ODS) column (YMC-Pack ODS-A, 100 × 4.6 mm).

Biotransformation The sapogenin 1 or 2 (5 µmol in 50 µl of dimethylsulfoxide) was incubated with rat liver S9 fraction (0.25 g liver eq) of male Wistar rat, pretreated with phenobarbital as a cytochrome P450 inducer, at 37 °C for 1 h in the presence of 0.5 mM NADPH, 5 mM glucose-6-phosphate and 5 mM MgCl2 in a final volume of 5.0 ml of 100 mM potassium phosphate buffer (pH 7.4). The metabolic reaction was quenched by addition of 15 ml of ice-cold methanol (MeOH). The metabolic fraction was separated by centrifugation and evaporated. The residue was dissolved into 1 ml of MeOH, and then subjected to HPLC and MS analyses.

Results and Discussion

A typical HPLC chromatogram in the case of 1 is shown in Fig. 2. The amount of total substances changed was estimated to be 40% of 1 and 20% of 2. As expected, 20,24-epoxides 3 and 4 were liberated as the metabolites of 1. The identification of the metabolites was performed by comparison of FAB-MS, EI-MS and retention times of HPLC with those of authentic samples derived from 1 by oxidation with m-chloroperbenzoic acid.5) In the same manner, 20,24-epox-
ide 5 was identified in the case of 2, but the formation of 6 was ambiguous because it overlapped with other minor peaks in the HPLC. The formation of 24S-epimer was much higher (3: ca. 40% and 5: 50% of the total metabolites) than that of 20R-epimer (4: ca. 4%). Dammarenediol II (8), which is a 12-deoxy analogue of 1 and 2 isolated from dammar resin, was also tested under the same conditions, but the corresponding 20,24-epoxides were not detected, suggesting that the presence of 12-hydroxy group is significant to form 20,24-epoxide in this metabolism.

In subsequent experiments, we observed that the formation of 3 from 1 is predominant in the S9 fraction from untreated rats rather than the S9 from phenobarbital pretreated rats. Details of these enzymatic properties of the formation of 3 catalyzed by rat liver microsomal cytochrome P450 will be presented elsewhere.

Thus, it was confirmed that the sapogenins could easily be transformed to their 20,24-epoxides with rat liver microsomes. This is the first certification of the metabolites of ginseng sapogenins. To establish the role of this biotransformation pathway, comparative studies on the biological activities of the metabolites 3—6 with those of 1 and 2 are required. Further experiments of the other metabolites from sapogenins and those with respect to the prosapogenins are in progress.

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References and Notes